Diversity of Owl Endemic Minahasa, Otus manadensis, through DNA Barcoding techniques

Hapry F.N. Lapian[1♥], Jane S.I.T. Onibala[1], Sylvia Laatung[1]
[1]Department of Animal Sciencey, Faculty of Animal Science, Sam Ratulangi University. Kel. Bahu, Kec. Malalayang, Manado,
♥corresponding mail: haprylapian@unsrat.ac.id

Abstract. In this paper, the results of mitochondrial COI sequences extracted from the *Otus manadensis,* typical owl of Minahasa, are reported and compared with COI sequences from other parts of the world. The results of phylogenetic analysis reveal that there are three distinctive groups of typical owls: (i) the *Otus manadensis* (ii) mainland Asia, and (iii) African owls. The phylogenetic topology also highlights that there is similar clade between *Otus manadensis* and those from the Philippines, as well as a significant divergence between *Otus manadensis* and those of African species (*Otus hartlaubi* and *Otus feae*). Therefore, it can be said that *Otus manadensis* occupies a unique position in the classification of owls. These results also allow further research to better assess the taxonomic status of typical owls according to its peculiarities that may not be identified through traditional taxonomy.

Key words: Aves, Minahasa Owls*, Otus manadensis*, COI, taxonomy

INTRODUCTION

The taxonomy and systematics of birds are fascinating to study due to their ecological roles and positions within ecosystems (Whelan et al., 2015). Owls, belonging to the order *Strigiformes*, are a particularly well-studied group, distinguished by their binocular vision, predatory adaptations, and nocturnal behavior. Owls show a wide distribution and consist of more than 240 species grouped in two families: Tytonidae (barn owls) and Strigidae (typical owls). In North Sulawesi, the Owls is identified as *Tyto inexspectata* and *Otus manadensis*. The International Union for Conservation of Nature (IUCN), the global authority on natural resource conservation, classifies *Tyto inexspectata* as Vulnerable (VU) (BirdLife International, 2016a) and *Otus manadensis* as Least Concern (LC) (BirdLife International, 2016b) on the Red List of Threatened Species. These classifications highlight the urgency of implementing conservation measures, particularly to determine their genetic diversity and phylogenetic relationships.

Research on the genetic relationships and phylogenetic positions of various owl species has been extensively conducted. Studies focusing on the phylogeny of the *Strigiformes* order, particularly using mitochondrial DNA sequences, have

identified several clades among owl species (Wink et al., 2011; Omote et al., 20.3; Xu et al., 2016). Additionally, investigations utilizing non-coding control regions, such as the COI gene, have successfully established classifications within owl groups (Sun et al., 2020; Zhang et al., 2016; Xu et al., 2016; Hengjiu et al., 2016). Furthermore, more comprehensive analyses involving complete DNA sequences have also been undertaken to explore the phylogenetic relationships of owls (Yu et al., 2021; Kang et al., 2018; Omote et al., 2013; Xiao et al., 2003; Sun et al., 2016; Xu et al., 2016; Liu et al., 2014).

The *Otus manadensis* population in North Sulawesi is facing a concerning decline, emphasizing the need for effective conservation measures. However, before implementing conservation strategies, a deeper understanding of the species diversity is essential. Accurate species identification, particularly through molecular approaches, plays a crucial role in this process.

Analyzing the genetic variation of *Otus manadensis*, an endemic species of Minahasa, is crucial as it provides accurate data to support species preservation through the development of targeted conservation programs (Enriques et al., 2017; Grzywaczewski et al., 2024; Solonen, 2024). Conducting genetic analysis to explore the phylogenetic relationships between *Otus manadensis* and other owl species from various regions is equally important. Utilizing mitochondrial DNA (mtDNA) is an effective approach for assessing the genetic diversity of this distinctive Minahasan species.

The mitochondrial cytochrome c oxidase subunit I (COI) gene is highly esteemed as an effective molecular tool for identifying species, often referred to as "DNA barcoding" specifically for birds (Aliabadian et al., 2013; Huang and Ke, 2014; Huang et al., 2015; Chaves et al., 2015; Barreira et al., 2016; Tizard et al., 2019; Colihueque et al., 2021; Pulgarín-R et al., 2021). It is the standard for animal DNA barcoding (Pentinsaari et al., 2016; Heller et al., 2018; Antil et al., 2023; Alam et al., 2024) and is increasingly utilized in DNA metabarcoding studies (Porter and Hajibabaei, 2018; Ruppert et al., 2019). This gene also plays a key role

in advancing the understanding of taxonomic relationships among species (Andújar et al., 2018; Macher et al., 2018; Deagle et al., 2014; Creedy et al., 2022). The COI gene comprises a 648-base pair segment of the mitochondrial genome, offering high accuracy in distinguishing animal species due to substantial sequence variation between closely related species. Its effectiveness in defining species boundaries has led to its widespread adoption for species identification, with researchers relying on a database of sequences linked to taxonomically verified voucher specimens.

This study involved sequencing a 648-base pair segment of the COI gene from specimens of the *Otus manadensis*. The primary objective was to analyze the COI sequences to assess the genetic diversity within this endemic species. Given the scarcity of information on genetic divergence in Otus manadensis using the COI gene, this research aims to provide essential insights that could significantly contribute to a clearer taxonomic classification of this unique species. The findings from this analysis will be instrumental in guiding conservation strategies and enhancing our understanding of the genetic relationships and evolutionary history of Otus manadensis.

MATERIALS AND METHODS

Ethics statement

The present study was approved by the Animal Ethics Commission of Sam Ratulangi University.

Samples collection and DNA extraction

Blood samples from four healthy adult Manguni owls were collected from various locations in Minahasa Regency between April and June 2023. Each owl was physically restrained by experts for the procedure. A total of 1 mL of blood was drawn from the jugular or ulnar vein into tubes containing ethylenediaminetetraacetic acid (EDTA), and the samples were immediately

stored at 4°C. Genomic DNA was extracted from the total blood samples using the Genomic DNA Mini Kit (Geneaid, GP100 catalog) following the provided instructions. A 100 µL portion of each blood sample was processed for DNA extraction from the cells.

PCR and sequencing

The PCR kit uses MyTaq™ HS Red Mix (Bioline). COI sequences were amplified using the primer pair of LCO1490 (5'- GGTCAACAAATCATAAAGATATTGG -3') and HC02198 (5'- TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994). PCR amplification was carried out in Each 40 µl PCR reaction contained 1x MyTaq™ HS Red Mix (Bioline), 1.5 nmol of each primer, and 2 µl of sample DNA. Heating was carried out using a TPersonal thermocycler (Biometra) with an initial denaturation temperature of 95℃ (3 minutes) and followed by 35 cycles: Denaturation 95℃ (20 seconds) - Primer attachment 50℃ (20 seconds) - DNA elongation 72℃ (30 seconds). PCR results were observed using 0.8% agarose gel electrophoresis. PCR success was indicated by a single band of 709 bp.

Sequencing

Sequencing was performed both ways using each PCR primer by First Base C.O. (Malaysia). Chromatograms were edited using Geneious Primer v2023.1.2. Alignment uses the MUSCLE algorithm (Edgar, 2004).

Phylogenetic and sequence divergence analyses

The phylogenetic trees, conducted in Mega 11 (Stecher et al., 2020; Tamura et al., 2021), was constructed using the Neighbor-Joining (Saitou and Nei, 1987). The optimal tree is shown.

RESULTS AND DISCUSSION

This study analyzed 10 nucleotide sequences from Minahasa's owl, *Otus manadensis,* specimens along with seven representative sequences from other regions. The alignment of the COI gene sequences consisted of 537 positions. All nine sequences of the *Otus manadensis* specimens were retrieved from GenBank (http://www.ncbi.nlm.nih.gov/Genbank) (Table 1). The COI gene sequence alignment, including sequences from *Otus manadensis* and representative specimens from other regions, was examined using the Neighbor-Joining (NJ) method, which identified two primary clusters (Figure 1).

Table 1 List of eight specimens owls from GenBank

Species	**Country/Continent**	**Accesien numbers**
Otus longicornis	The Phillipine	OM937294.1
Otus madagascariensis	Madagascar	OM937295.1
Otus elegans	Japan	AB842983.1
Otus scops	Europe	KY471456.1
Otus sunia	Oriental	ON016106
Otus scops cyprius	Cyprus	KT803674
Otus hartlaubi	Africa	OQ272185.1
Otus feae	Equatorial Guinea	OQ272267.1

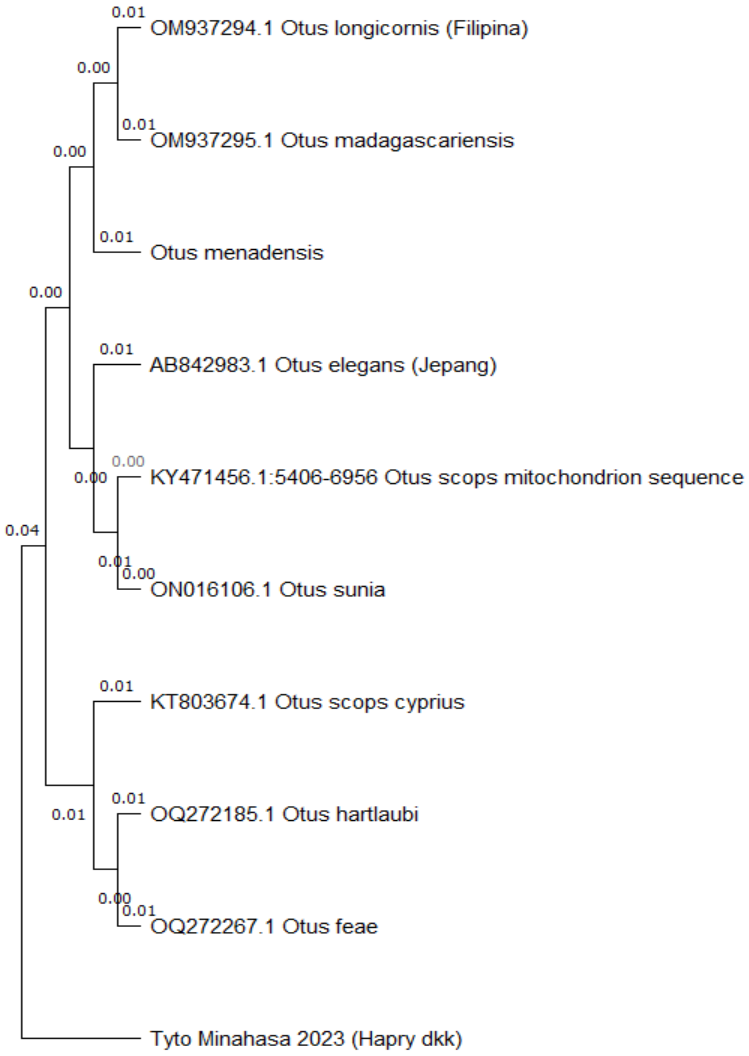

Figure 1 The tree was inferred using the Neighbor-Joining method [Sitou and Nei, 1987]. This analysis involved 10 nucleotide sequences with Tyto Minahasa as outgroup.. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 537 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [Tamura et al., 2021] [Stercher et al., 2020]

The phylogenetic analysis of *COI* sequences for *Otus manadensis* in this study revealed distinct clustering patterns, consistent with previous findings. Earlier research has demonstrated a clear phylogenetic separation between the Common Barn Owl (*Tyto alba*) and the typical owls (*Otus manadensis*), which strongly support significant divergence between the *Otus* and *Tyto* clades.

The results further corroborate genetic differentiation among *Otus manadensis* populations from Continental Asia, Africa, and southern Europe, as reported in earlier studies (Wink et al., 2011; Aliabadian et al., 2013). Notably, the phylogenetic topology also highlights a clear separation between *Otus manadensis;* morphologically similar owls from the Philippines and African species (*Otus hartlaubi* and *Otus feae*). Cumulative molecular evidence supports the classification of these clades as distinct species. However, further integrative studies encompassing morphological, molecular, and acoustic data are required to fully elucidate these taxonomic distinctions.

The genetic distances observed among populations of typical owls from regions outside Minahasa, ranging from 0.06% to 3.5% (Table 2), are consistent with expected patterns of genetic differentiation. These findings highlight significant genetic divergence in *Otus manadensis* across its geographic range, with pronounced differentiation among populations from widely separated regions. The data suggest that the Manguni Minahasa population may represent a distinct species, separate from other members of the *Otonini* tribe. This evidence supports the classification of Manguni Owls as a separate species, and based on the observed genetic divergence, the designation of *Otus manadensis* as a distinct species is proposed.

The sequence variation observed in *Otonini* tribes, including *Otus manadensis* from Minahasa, compared to populations from other continents, is consistent with the average intraspecific genetic distance reported for birds, which typically ranges between 0.23% and 0.43% (Aliabadian et al., 2013; Hebert et al., 2004; Kerr et al., 2007, 2009). These values are comparable to those documented for other owl species analyzed using *COI* sequence data, which report an average genetic distance of less than 2% (Nijman and Aliabadian, 2013).

Table 2. Estimates of Evolutionary Divergence between Sequences

		1	2	3	4	5	6	7	8	9	10
1	OM937294.1_Otus_longicornis_(Filipina)										
2	Otus_menadensis	0,0222									
3	OM937295.1_Otus_madagascariensis	0,0203	0,0227								
4	AB842983.1_Otus_elegans_(Jepang)	0,0251	0,0223	0,0243							
5	KY471456.1:5406-6956_Otus_scops_mitochondrion_sequence	0,0267	0,0270	0,0196	0,0220						
6	ON016106.1_Otus_sunia	0,0270	0,0276	0,0198	0,0226	0,0006					
7	KT803674.1_Otus_scops_cyprius	0,0341	0,0292	0,0339	0,0337	0,0320	0,0326				
8	OQ272185.1_Otus_hartlaubi	0,0315	0,0310	0,0358	0,0311	0,0334	0,0340	0,0174			
9	OQ272267.1_Otus_feae	0,0297	0,0318	0,0339	0,0324	0,0315	0,0322	0,0187	0,0133		
10	Tyto_Minahasa_2023_(Hapry_dkk)	0,1253	0,1319	0,1295	0,1277	0,1304	0,1317	0,1331	0,1317	0,1278	

Previous studies have often attributed high levels of sequence divergence to potential taxonomic artifacts, emphasizing the need for further taxonomic scrutiny. For example, Hebert et al. (2004) reported intraspecific divergences ranging from 3.7% to 7.2% in four of 260 North American bird species, while Kerr et al. (2009) documented sequence divergences exceeding 2.4% in 13 of 389 bird species from Argentina. These patterns of divergence have been linked to factors such as disjunct ranges, ecological variation, non-migratory behavior, and rapid speciation (Kerr et al., 2009).

In the case of *Otus manadensis*, geographical barriers to gene flow among populations across continents are anticipated, given its predominantly non-migratory or short-distance migratory behavior (Matics, 2003), which likely facilitates genetic divergence. However, contrary to expectations, all *Otus manadensis* analyzed in this study exhibited significant genetic similarity, with no notable divergences detected. This observation underscores the need for further analyses using additional molecular markers to confirm the findings.

In conclusion, this study highlights significant intraspecific COI sequence divergence in Otus manadensis across its geographic range, exceeding previous

records for Strigiformes. The findings suggest that geographic barriers and ecological diversity contribute to genetic differentiation, despite the species' non-migratory nature. Further research integrating ecological, morphological, and genetic data is necessary to understand the evolutionary processes and reassess the taxonomic classification of Minahasa owl populations. This comprehensive approach will aid in accurate species identification and conservation efforts.

REFFERENCES

Aliabadian M, Beentjes KK, Roselaar CS, van Brandwijk H, Nijman V, Vonk R. (2013) DNA barcoding of Dutch birds. Zookeys. 2013 Dec 30;(365):25-48. doi: 10.3897/zookeys.365.6287. PMID: 24453549; PMCID: PMC3890669.

Alam, M., Abbas, K., Usmani, N., Raza, M. T., Bhat, M. R., Mustafa, M. & Husain, A. (2024). A comprehensive review on DNA barcoding for species identification across diverse taxa. Munis Entomology & Zoology, 19 (2): 1057-1072

Andújar C, Arribas P, Yu DW, Vogler AP, Emerson BC. (2018). Why the COI barcode should be the community DNA metabarcode for the metazoa. Mol Ecol. 2018 Oct;27 (20):3968-3975. doi: 10.1111/mec.14844. Epub 2018 Sep 17. PMID: 30129071.

Antil, S., Abraham, J.S., Sripoorna, S. *et al.* (2023). DNA barcoding, an effective tool for species identification: a review. *Mol Biol Rep* **50**, 761–775 (2023). https://doi.org/10.1007/s11033-022-08015-7

Barreira AS, Lijtmaer DA, Tubaro PL. (2016). The multiple applications of DNA barcodes in avian evolutionary studies. Genome. 2016 Nov;59(11):899-911. doi: 10.1139/gen-2016-0086. Epub 2016 Oct 6. PMID: 27824508.

BirdLife International, (2016a). *Tyto inexspectata.* The IUCN Red List of Threatened Species 2016.e.T22688441A93197763. http://dx.doi.org/10.2305/IUCN.UK.2016-3.RLTS.T22688441A93197763.en.

BirdLife International, (2016b). *Otus manadensis*. The IUCN Red List of Threatened Species 2016: e.T62290590A95195305. http://dx.doi.org/10.2305/IUCN.UK.20163.RLTS.T62290590A95195305.en.

Chaves BR, Chaves AV, Nascimento AC, Chevitarese J, Vasconcelos MF, Santos FR. (2015) Barcoding Neotropical birds: assessing the impact of nonmonophyly in a highly diverse group. Mol Ecol Resour. 2015 Jul;15(4):921-31. doi: 10.1111/1755-0998.12344. Epub 2014 Dec 14. PMID: 25417731.

Colihueque N, Gantz A, Parraguez M. (2021). Revealing the biodiversity of Chilean birds through the COI barcode approach. Zookeys. 2021 Feb 11;1016:143-161. doi: 10.3897/zookeys.1016.51866. PMID: 33628081; PMCID: PMC7892532.

Creedy TJ, Andújar C, Meramveliotakis E, Noguerales V, Overcast I, Papadopoulou A, Morlon H, Vogler AP, Emerson BC, Arribas P. (2022). Coming of age for COI metabarcoding of whole organism community DNA: Towards bioinformatic harmonisation. Mol Ecol Resour. 2022 Apr;22(3):847-861. doi: 10.1111/1755-0998.13502. Epub 2021 Sep 30. PMID: 34496132; PMCID: PMC9292290.

Deagle BE, Jarman SN, Coissac E, Pompanon F, Taberlet P. (2014). DNA metabarcoding and the cytochrome c oxidase subunit I marker: not a perfect match. Biol Lett. 2014 Sep;10(9):20140562. doi: 10.1098/rsbl.2014.0562. PMID: 25209199; PMCID: PMC4190964.

Edgar RC. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 2004 Mar 19;32(5):1792-7. doi: 10.1093/nar/gkh340. PMID: 15034147; PMCID: PMC390337.

Enriques, P.L., K. Eisermann, H. Mikkola, J.C. Motta-Junior. (2017). A review of the systematics of Neotropical Owl (Strigiformes). P.L Enriques (ed.), *Neotropical Owls Diversity and Conservation.* DOI 10.1007/978-3-31957108-9_2.

Grzywaczewski, G., Morelli, F., & Skórka, P. (2024). Little Owl Aggression and Territory in Urban and Rural Landscapes. *Animals : an open access journal from MDPI*, *14*(2), 267. https://doi.org/10.3390/ani14020267

Hebert PDN, Stoeckle MY, Zemlak TS, Francis CM. (2004). Identification of birds through DNA barcodes. Plos Biology 2: 1657–1663. doi: 10.1371/journal.pbio.0020312

Heller P, Casaletto J, Ruiz G, Geller J. (2018). A database of metazoan cytochrome c oxidase subunit I gene sequences derived from GenBank with CO-ARBitrator. Sci Data. 2018 Aug 7;5:180156. doi: 10.1038/sdata.2018.156. PMID: 30084847; PMCID: PMC6080493.

Hengjiu, T., Jianwei, J., Shi, Y., Zhiming, Z., Laghari, M. Y., Narejo, N. T., & Lashari, P. (2016). Complete mitochondrial genome of Eagle Owl (Bubo bubo, Strigiformes; Strigidae) from China. *Mitochondrial*

DNA. Part A, DNA mapping, sequencing, and analysis, *27*(2), 1455–1456. https://doi.org/10.3109/19401736.2014.953090

Huang ZH, Ke DH. (2014). DNA barcoding and evolutionary relationships of the Phasianidae family in China. Genet Mol Res. 2014 Sep 12;13(3):7411-9. doi: 10.4238/2014.September.12.7. PMID: 25222240.

Huang ZH, Tu FY, Liao XJ. (2015). DNA barcoding and phylogenetic relationships of genera Picoides and Dendrocopos (Aves: Picidae). Genet Mol Res. 2015 Dec 28;14(4):18370-5. doi: 10.4238/2015.December.23.24. PMID: 26782484.

Kang, H., Li, B., Ma, X., & Xu, Y. (2018). Evolutionary progression of mitochondrial gene rearrangements and phylogenetic relationships in Strigidae (Strigiformes). *Gene*, *674*, 8–14. https://doi.org/10.1016/j.gene.2018.06.066

Kerr KCR, Lijtmaer DA, Barreira AS, Hebert PDN, Tubaro PL. (2009). Probing evolutionary patterns in Neotropical birds through DNA barcodes. PLoS ONE 4: e4379. doi: 10.1371/ journal.pone.0004379

Kerr KCR, Stoeckle MY, Dove CJ, Weigt LA, Francis CM, Hebert PDN. (2007). Compre¬hensive DNA barcode coverage of North American birds. Molecular Ecology Notes 7: 535–543. doi: 10.1111/j.1471-8286.2007.01670.x

Liu, G., Zhou, L., & Gu, C. (2014). The complete mitochondrial genome of Brown wood owl Strix leptogrammica (Strigiformes: Strigidae). *Mitochondrial DNA*, *25*(5), 370–371. https://doi.org/10.3109/19401736.2013.803540

Macher JN, Vivancos A, Piggott JJ, Centeno FC, Matthaei CD, Leese F. (2018). Comparison of environmental DNA and bulk-sample metabarcoding using highly degenerate cytochrome c oxidase I primers. Mol Ecol Resour. 2018 Nov;18(6):1456-1468. doi: 10.1111/1755-0998.12940. Epub 2018 Sep 9. PMID: 30129704.

Matics R. (2003). Direction of movements in Hungarian Barn Owls (Tyto alba): gene flow and barriers. Diversity and Distributions 9: 261–268. doi: 10.1046/j.1472-4642.2003.00018.x

Nijman V, Aliabadian M. (2013). DNA Barcoding as a Tool for Elucidating Species Delinea¬tion in Wide-ranging Species as Illustrated by Owls (Tytonidae and Strigidae). Zoological Science 30: 1005–1009. doi: 10.2108/zsj.30.1005

Omote, K., Nishida, C., Dick, M. H., & Masuda, R. (2013). Limited phylogenetic distribution of a long tandem-repeat cluster in the mitochondrial control region in Bubo (Aves, Strigidae) and cluster variation in

Blakiston's fish owl (Bubo blakistoni). *Molecular phylogenetics and evolution*, *66*(3), 889–897. https://doi.org/10.1016/j.ympev.2012.11.015

Pentinsaari, M., Salmela, H., Mutanen, M. *et al.* (2016). Molecular evolution of a widely-adopted taxonomic marker (COI) across the animal tree of life. *Sci Rep* **6**, 35275 (2016). https://doi.org/10.1038/srep35275

Porter TM and Hajibabaei M. (2018). Over 2.5 million COI sequences in GenBank and growing. PLoS One. 2018 Sep 7;13(9):e0200177. doi: 10.1371/journal.pone.0200177. PMID: 30192752; PMCID: PMC6128447.

Pulgarín-R PC, Olivera-Angel M, Ortíz L, Nanclares D, Velásquez-Restrepo S, Díaz-Nieto JF. (2021) DNA barcodes of birds from northern Colombia. Biodivers Data J. 2021 May 21;9:e64842. doi: 10.3897/BDJ.9.e64842. PMID: 34084068; PMCID: PMC8163714.

Ruppert, K.M., Richard J. Kline, Md Saydur Rahman, (2019). Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA, Global Ecology and Conservation, Volume 17, 2019, e00547, ISSN 2351-9894, https://doi.org/10.1016/j.gecco.2019.e00547.

Saitou N. and Nei M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**:406-425.

Solonen, T. (2024). Owls' Responses to Environmental Challenges. *Animals*, *14*(6), 880. https://doi.org/10.3390/ani14060880

Stecher G., Tamura K., and Kumar S. (2020). Molecular Evolutionary Genetics Analysis (MEGA) for macOS. *Molecular Biology and Evolution* 37:1237-1239.

Sun, C. H., Liu, H. Y., Min, X., & Lu, C. H. (2020). Mitogenome of the little owl Athene noctua and phylogenetic analysis of Strigidae. *International journal of biological macromolecules*, *151*, 924–931. https://doi.org/10.1016/j.ijbiomac.2020.02.238

Sun, X., Zhou, W., Sun, Z., Qian, L., Zhang, Y., Pan, T., & Zhang, B. (2016). The complete mitochondrial genome of Glaucidium brodiei (Strigiformes: Strigidae). *Mitochondrial DNA. Part A, DNA mapping, sequencing, and analysis*, *27*(4), 2508–2509. https://doi.org/10.3109/19401736.2015.1036252

Tamura K., Stecher G., and Kumar S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11.

Molecular Biology and Evolution https://doi.org/10.1093/molbev/msab120.

Tizard J, Patel S, Waugh J, Tavares E, Bergmann T, Gill B, Norman J, Christidis L, Scofield P, Haddrath O, Baker A, Lambert D, Millar C. (2019). DNA barcoding a unique avifauna: an important tool for evolution, systematics and conservation. BMC Evol Biol. 2019 Feb 11;19(1):52. doi: 10.1186/s12862-019-1346-y. PMID: 30744573; PMCID: PMC6369544.

Wink, Michael & El-Sayed, Abdelaziz & Sauer-Gürth, Hedwig. (2011). Molecular Phylogeny of Owls (Strigiformes) Inferred from DNA Sequences of the Mitochondrial Cytochrome b and the Nuclear RAG-1 gene. Ardea. 97. 581591. 10.5253/078.097.0425.

Xiao, B., Ma, F., Sun, Y., & Li, Q. W. (2006). Comparative analysis of complete mitochondrial DNA control region of four species of Strigiformes. *Yi chuan xue bao = Acta genetica Sinica*, *33*(11), 965–974. https://doi.org/10.1016/S0379-4172(06)60131-5

Xu, P., Li, Y., Miao, L., Xie, G., & Huang, Y. (2016). Complete mitochondrial genome of the Tyto longimembris (Strigiformes: Tytonidae). *Mitochondrial DNA. Part A, DNA mapping, sequencing, and analysis*, *27*(4), 2481–2482. https://doi.org/10.3109/19401736.2015.1033708

Yu, J., Liu, J., Li, C., Wu, W., Feng, F., Wang, Q., Ying, X., Qi, D., & Qi, G. (2021). Characterization of the complete mitochondrial genome of *Otus lettia*: exploring the mitochondrial evolution and phylogeny of owls (Strigiformes). *Mitochondrial DNA. Part B, Resources*, *6*(12), 3443–3451. https://doi.org/10.1080/23802359.2021.1995517

Zhang, Y., Song, T., Pan, T., Sun, X., Sun, Z., Qian, L., & Zhang, B. (2016). Complete sequence and gene organization of the mitochondrial genome of Asio flammeus (Strigiformes, strigidae). *Mitochondrial DNA. Part A, DNA mapping, sequencing, and analysis*, *27*(4), 2665–2667. https://doi.org/10.3109/19401736.2015.1043538